

The University of Chicago

THE EFFECT OF VITAMIN B DEFICIENCY
DURING NURSING ON SUBSEQUENT
LEARNING IN THE RAT

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PSYCHOLOGY

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By
KARL SCHOFIELD BERNHARDT

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I. INTRODUCTION

The purpose of this investigation was to discover whether a deficiency in vitamin B during the nursing period had any noticeable effect on subsequent learning in the rat. Vitamin B is indispensable for normal growth. The rat is born in an undeveloped state, and as the nervous system is still under development during the first few weeks after birth, it was thought that a lack of vitamin B during this period would result in a development of the nervous system less than normal, and therefore probably produce a learning ability below the normal. The use of human subjects in such an experiment as this is almost impossible due to the complexity of the factors involved, while the rat is an ideal subject.

Physiological effects of vitamin deficiency have received a great deal of attention. Sure, Kirk and Smith (17) have shown that vitamin B deficiency reduces the appetite causing a lessened food intake and loss of weight. It was thought previously that the effect of vitamin B deficiency was principally on the nervous system but McCarrison, as quoted by McCollum and Simmonds (11) demonstrated that there were functional and degenerative changes in every tissue of the body, and that the effect on the brain tissue was the least serious. Jackson (4) has shown that

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The experimental work was done in the Departments of Psychology and Paediatrics at the University of Toronto.

Grateful acknowledgment is made to Professor Harvey A. Carr of the University of Chicago for his advice and criticism.

the brain-weight of rats whose body-weight had been held constant from three to eight weeks of age remained about constant while other organs showed a tendency to growth, and still others showed a loss of weight. Jackson and Stewart (5, 6) refed rats after early underfeeding and found that although other parts of the body returned to normal weight, the brain had been affected in some way by the inanition so that its later growth on ample refeeding was retarded behind the body as a whole in its ultimate growth in weight. Because of the relative immaturity of the nervous system at birth, it is possible that vitamin deficiency at this early period might have serious effects because of its interference with the normal formative processes.

Few studies have investigated directly the relationship of diet and learning, and all such studies have employed a food incentive in the learning situation. Anderson and Smith (1) studied the effect of qualitative and quantitative stunting of rats on their maze learning. Stunted rats were found to be superior to normal rats in the maze but realimentation brought the stunted rats close to the normal both in weight and learning ability. One very probable explanation of this result is a difference in motivation between the groups, as a food incentive maze was used and the stunted rats were probably more highly motivated. The results of this experiment would seem to indicate that the use of a learning situation in which food motivation is used is a doubtful procedure in experiments dealing with the relation of diet and learning ability.

Frank (2) investigated the effect of a rickets-producing diet on the learning ability of rats. Rats with active rickets learned the maze in fewer trials than normal rats and with fewer errors but consumed more time in learning. This difference disappeared with realimentation. Miss Frank concluded that if differences in learning ability do exist in normal, rachitic and underweight rats the usual maze technique is inadequate for their demonstration.

Fritz (3) published the results of an experiment in which he studied the effect of vitamin B deficiency and an unfavorable salt mixture on maze performance. The deficiency in diet took

place with adult rats and during the time of the maze tests. The deficient animals showed consistently poorer learning scores as a group. There were marked individual differences in the ability to withstand the effects of faulty diets.

The work of Maurer and Tsai (8, 9, 10) is more closely related to the present investigation. They were interested in the effects of a vitamin B deficiency during the nursing period upon later learning. The experimental group of rats were nursed by mothers which were fed a B-free diet, and this group was compared with a control group which were nursed by mothers on a normal diet. The authors state (9, page 53):

After the depleted animals were weaned they were given a week of vitamin rich diet so that their bodyweight was brought nearer to that of the control animals. On the other hand when the control animals were 60 days old they were fed on the B-free diet for a few days to slightly check their rate of growth without depleting them. This makes the two groups equal in their strength of incentive.

The maze pattern used was designed by Professor Carr of the University of Chicago. The incentive employed in this maze was a vitamin concentrate, rich in vitamins B and E. It would seem that this manipulation of the diet previous to the learning trials in order to try to equate the weights of the groups had an unknown and uncontrolled effect on subsequent learning. There is some doubt as to whether the two groups were "equal in their strength of incentive." The results of the experiment indicate reliable differences in learning ability, the deficient rats being consistently poorer as a group than the normal controls. An inspection of the individual records shows that there was a certain amount of overlapping of the groups, and that possibly the effect of the deficiency was more serious with some rats than with others. The general results of their experiment are important enough to be verified.

This short survey of the literature on the relation of diet to learning indicates that there is at least some effect on learning in rats when there is a deficiency in the diet, and that probably this effect is greater when the deficiency occurs early in the life of the animal.

The present investigation was designed to verify the conclusions of Maurer and Tsai's experiment under different conditions, namely, an escape-from-water incentive in place of hunger motivation, a different maze pattern, and also with a very different type of learning problem, namely, multiple choice. As stated above, Maurer and Tsai, as well as other investigators of learning of rats with deficient diets, employed the usual food-incentive mazes. Because we believe that it is impossible to know the specific effects of dietary differences on hunger motivation it was thought best to use some other form of motivation in the present study. Preliminary experimentation indicated that escape-from-water was possibly the best substitute. This form of motivation was suggested by the work of McDougall (12). During the period of this preliminary experimentation with the escape-from-water incentive Ruch (14) published an article in which he demonstrated that although food was more effective than water as an incentive the difference between the two was slight. He writes,

It is concluded that the escape-from-water method of motivating animal learning is well adapted to maze procedures. This method has particular value where it is necessary to employ motivation which is independent of the hunger drive.

II. PROCEDURE

The diets, breeding, care and feeding of the animals used in this experiment were taken care of by the staff of the Department of Paediatrics of the University of Toronto.

1. Diet and subjects

Litters were assigned at birth alternately to the control and experimental groups. Each litter was reduced at birth to six rats. Maurer and Tsai did not equate the size of the litters in this way, but it was thought necessary to follow this procedure so as to keep as constant as possible the amount of food consumed. The mothers of the experimental group were fed on a B-free diet until the litters were removed. The mothers of the control group were given the same B-free diet with 6 per cent brewers' yeast

added. When the litters were four weeks old they were taken from their mothers and both groups placed on the same diet, namely the B-free diet plus 6 per cent yeast. This diet was used for all animals during the rest of the experiment. The rats were allowed to eat as much as they desired. The only difference in the diet of the two groups was, then, that vitamin B complex had been removed from the food of the nursing mothers of the experimental group during the period of four weeks when they were nursing the litters, while the mothers of the control group were given a normal diet. Sure (16) has shown that the quantity of vitamin B in the milk of the nursing mother rat is proportional to the vitamin B content of the mother's diet, so it was assumed that by removing vitamin B from the diet of the mother we were depleting the litters as well. The depletion of vitamin B during this period of four weeks may not have been complete or uniform for all animals of the experimental group. There might be a certain amount of storage of vitamin B in the mothers and this storage might vary from rat to rat. This fact of vitamin storage is indicated by the work of Osborne and Mendel (13) who conclude that, "when an adequate supply of vitamin B is lacking in the diet the store of this factor in the liver tissue, where it is ordinarily found in abundance becomes largely depleted." However, this does not prove that this storage can be passed on to the litters by the mother. McCollum and Simmonds (11, page 412) state that "vitamins A and B pass into the mother's milk only when they are present in the diet of the mother." If this statement is true then the experimental animals did not receive any vitamin B from their mothers during the first four weeks of their life. It is possible, however, that the young rats themselves may have had a certain amount of storage of vitamin B when they were born as their mothers had been fed on a normal diet previous to their birth. There is the further factor of individual differences in the ability to withstand dietary deficiencies. That the experimental group were affected by the vitamin B deficiency is evident from the facts that there was a higher mortality rate and a lower average weight in the experimental group than in the control group.

All rats used in the experiment were from the breeding stock of the Department of Paediatrics of the University of Toronto. The following is a summary of the number of rats used:

Experimental (deficient) group

	<i>rats</i>
13 litters born, a total of	96
Reduced at birth to a total of	76
Died during the first four weeks	32
Died after four weeks	7
Learning records for 24 females, 13 males—total	37

Control (normal) group

13 litters born, a total of	91
Reduced at birth to	73
Died during the first four weeks	22
Sick or died after four weeks	5
Learning records for 21 females, 25 males—total	46

The mortality among the B-deficient animals was considerably greater than among the control group, as only 37 out of 76 deficient rats survived for the learning tests while 46 out of 73 of the control group could be used. Unfortunately the sex ratio of the experimental group is not the same as that of the control group, but as the sex differences in maze learning are slight this was not considered serious. It was impossible to remedy this defect without some form of selection of subjects from the litters, but this was considered a more doubtful procedure than using the groups as they were constituted.

2. Weight records

A weight record was taken for each rat once a week during the course of the experiment. Table 1 shows the average weight of the two groups at birth and for the first three weeks, during which time the litters and not the individual animals were weighed. This table also includes the average weights of the two groups according to sex for every fourth week.

Figure 1 pictures the average weight of the two groups from birth to thirty weeks of age, separated according to sexes from the fourth week. It can be seen from the table and figure that the two groups were born with the same average weight, and

that the experimental group did not increase nearly as quickly as the control group during the first four weeks, but that the two groups increased at about the same rate after weaning. This difference in the rate of growth is taken as a clinical sign of vitamin B deficiency.

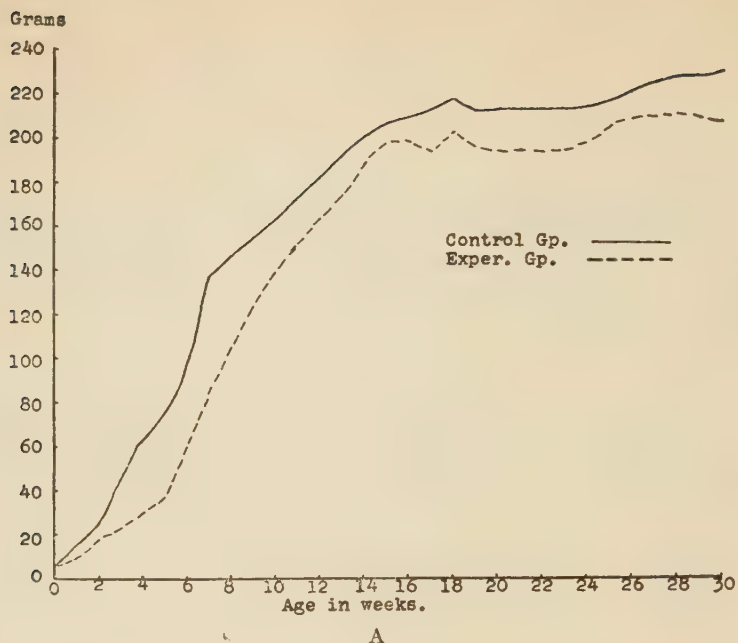
3. Activity records

It is possible that the difference in diet may have produced a difference in amount of activity and thus affect the learning records indirectly. It was thought advisable therefore to obtain

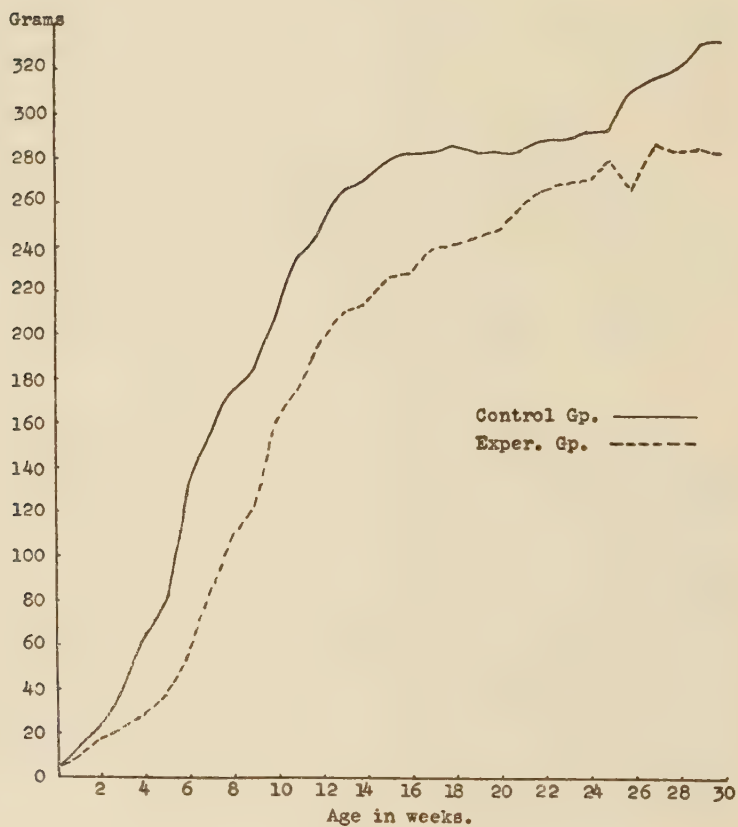
TABLE 1
Weight records in grams

AGE IN WEEKS	FEMALES		MALES		BOTH	
	Controls	Experimental	Controls	Experimental	Controls	Experimental
0					5.8	5.8
1					14.7	10.2
2					24.2	18.7
3					41.6	23.6
4	60.9	29.1	64.1	28.0		
8	144.7	104.1	175.5	108.8		
12	180.9	162.1	246.4	196.3		
16	206.9	197.6	281.8	227.7		
20	210.6	192.0	282.3	246.8		
24	211.9	196.3	291.1	268.3		
28	224.7	208.1	319.6	281.8		

some measure of the activity of the two groups. This was secured by the usual treadmill activity cage. Each rat was placed in this cage for twenty-four hours and the activity record taken as the number of revolutions of the cage in that time. The rats of the two groups were alternated so as to keep the age factor constant. Two such records were obtained for each animal about fifteen weeks apart. It was possible therefore to obtain a measure of the consistency of the activity records by correlating the first record with the second. The product moment coefficient obtained in this way was $+.74 \pm .03$. To further increase the reliability of the measures used the two records were averaged



A



B

FIG. 1. AVERAGE WEIGHT ACCORDING TO AGE

A.—Females. B.—Males

for each animal. The average scores of the two groups were as follows:

Average activity record of the experimental group...	833.6	P.E. 64.37
Average activity record of the control group	700.7	P.E. 53.75
Difference between the averages of the groups.....	132.9	P.E. 83.86
Difference over the P.E. of the difference.....	1.58	

There is then very little difference between the average activity records of the two groups, but what difference there is shows the experimental group to be slightly more active than the control group.

4. Preliminary swimming tests

The tank used in the preliminary trials as well as for all the learning problems was a galvanized iron tank 20 inches deep, 57 inches long, and 35 inches wide, with water 9 inches deep. The landing platform was a level surface 6 inches by 7 inches and 3 inches above the surface of the water with a wire mesh incline. For the preliminary trials half the width of the tank was used, the landing platform being placed at one end of the tank and the rat started at the other end. Time in seconds was taken from the time the rat was placed in the tank until he reached the landing platform. Each rat was given 20 trials as follows: one trial when the rat was seven weeks old, two trials the next day, three the next, four the next, five the next, and five the next. There was very little difference in the average time of the two groups on these trials—experimental group 172.2 seconds and the control group 172.6 seconds. This seems to indicate that the swimming ability and motivation of the two groups was probably about equal.

5. Learning problems

As has been previously stated the motivation used in all the learning problems was escape-from-water. Three problems were used: learning a water maze, relearning the same maze after two months, and a multiple choice problem in the water tank.

The maze pattern used is illustrated in figure 2. The maze had to be constructed in such a way as to minimize the possi-

bility of the rats clinging to the sides or partitions. This was achieved by having all corners of the maze right angles and making the pathways wide enough (6 inches) to preclude straddling. The tank in which the maze pattern fitted is described above. The water in the tank was kept at as constant a temperature as possible and approximately room temperature. Each rat was

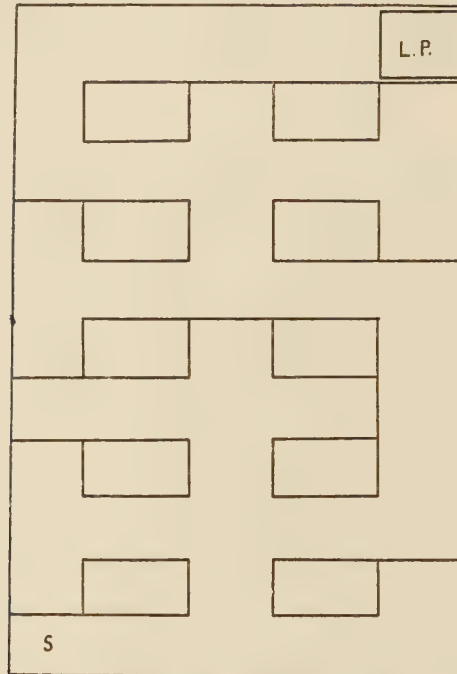


FIG. 2. PLAN OF THE WATER MAZE

L.P.—landing platform. S—start

dried on a towel before being returned to the cage and during the experiment none of the rats suffered from colds. Beginning at eight weeks of age the rats were given one trial every other day in the maze, and if the maze had not been learned in 32 trials they were given one trial every day from then on till completion. Half the experimental group and half the control group were run each day. Time in seconds was taken from the instant the rat

was dropped into the tank until he reached the landing platform. An error was recorded when the rat entered any blind alley with at least one-third of the body, that is, head and fore feet. A retrace was counted when the rat retraced at least one whole section of the maze, and only one retrace was counted no matter how far the rat retraced. Excess distance was recorded in terms

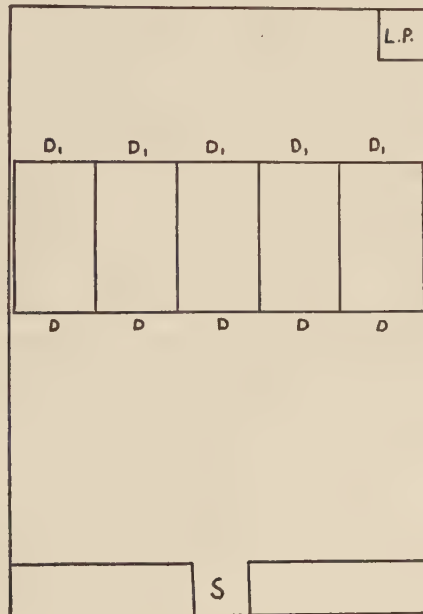


FIG. 3. PLAN OF THE MULTIPLE CHOICE APPARATUS

D—front doors of the choice compartments. D₁—back doors of the choice compartments. L.P.—landing platform. S—starting compartment.

of the number of maze sections entered by the rat going either forward or backward in the maze which were superfluous for a correct run. The maze was considered learned when the rat had had five errorless trials in succession.

The relearning trials were started when two months had elapsed since the rat had learned the maze. The same kinds of records were kept for this problem as for the learning trials. Five trials a day were given, the rat being started on the next trial a few

seconds after completing the former trial. The criterion of re-learning used was all five trials on one day without error.

The multiple choice problem was suggested by the work of Yerkes (18). A plan of the multiple choice apparatus used is sketched in figure 3. Escape-from-water motivation was again used. The problem selected was "the first on the left." Of the five compartments, three in a row were open each trial, a different three on each trial in a random order. If the rat entered the first open compartment on the left with head and fore feet the back door of that compartment would be opened and the rat allowed to swim to the landing platform. If the rat entered any other compartment the back door of that compartment would be left closed and the rat would have to retrace and enter another compartment and so on until he chose the correct one for that trial. Ten trials a day were given until the rat made no errors for one day. Several errors might be made on one trial. An error was counted as entering the wrong compartment. Time in seconds was taken for each trial.

III. RESULTS

1. Individual learning scores

The total scores for each individual animal of the two groups on the various measures in learning the maze, relearning the maze and learning the multiple choice problem are given in table 2 in order of magnitude for each measure. An inspection of this table shows that there are from 10 to 20 per cent of the experimental group whose records are poorer than any of the control group on each measure. It has been noted that there are great individual differences in the ability of rats to withstand the effects of dietary deficiencies, and it is suggested that this group of rats possibly suffered more from vitamin B deficiency than the rest of the experimental group.

2. Results for learning the maze

Table 3 shows the means, the probable errors of the means and the range of scores for the two groups on the various maze scores.

TABLE 2
Individual learning scores

LEARNING MAZE					RELEARNING MAZE					MULTIPLE CHOICE		
Trials	Time	Errors	Re-traces	Ex. Dist.	Trials	Time	Errors	Re-traces	Ex. Dist.	Trials	Time	Errors
Experimental group												
7	105	6	0	12	5	47	0	0	0	110	791	55
8	286	30	2	77	10	118	2	0	4	110	970	59
9	301	36	3	82	10	135	2	0	4	110	986	72
10	315	41	5	89	10	157	5	0	10	140	1026	80
13	323	45	6	100	15	194	6	0	12	150	1260	81
13	351	50	7	110	15	194	8	1	22	170	1286	98
14	389	52	9	129	15	197	9	1	24	170	1297	101
15	407	62	10	145	15	213	11	2	30	180	1453	110
15	472	62	10	156	15	229	13	2	34	180	1556	112
15	491	66	14	173	15	236	13	2	40	190	1635	126
15	533	73	14	180	15	259	15	2	41	210	1729	130
15	615	76	15	202	20	267	16	3	41	220	1730	137
16	655	95	15	240	20	279	16	4	41	230	1736	146
16	759	96	16	242	20	282	17	4	44	230	1737	149
16	897	96	17	243	20	282	17	4	47	230	1800	151
17	1153	147	20	345	20	317	24	4	59	230	1809	151
19	1325	155	27	396	20	339	26	5	73	250	1909	155
21	1493	163	30	444	20	368	27	5	80	280	1987	157
25	1518	185	30	460	20	411	29	6	82	290	2061	167
27	1560	199	32	541	25	419	31	6	92	290	2170	176
28	1593	217	32	546	25	422	38	7	95	300	2318	177
31	1931	222	32	558	30	452	38	7	97	310	2342	184
32	2037	223	35	558	30	491	38	8	101	310	2364	186
36	2130	229	38	571	30	539	46	8	108	320	2373	196
39	2260	254	41	590	30	558	51	8	123	320	2445	197
44	2339	266	48	618	30	640	60	9	146	320	2463	212
47	2374	266	50	654	35	802	62	9	156	330	2551	213
52	2389	308	51	779	45	816	71	10	179	340	2611	231
56	2501	314	55	799	45	841	72	10	184	360	2626	231
61	2659	317	57	829	45	1010	144	15	339	390	3020	232
65	2800	323	58	841	55	1055	147	17	347	400	3051	241
85	2952	337	60	869	85	1404	153	21	376	410	3129	248
91	3338	354	63	884	100	1775	166	23	468	420	3485	255
98	3512	477	65	1067	175	5340	382	87	973	450	3515	256
100	3926	488	67	1155	220	5615	443	127	1159	480	4020	258
150	4587	507	86	1220						490	4382	280
150	5818	588	109	1411						500	6785	290
Median. .25	1518	185	30	460	20	368	27	5	80	290	2061	167

TABLE 2—*Continued*

LEARNING MAZE					RELEARNING MAZE					MULTIPLE CHOICE		
Trials	Time	Errors	Retraces	Ex. Dist.	Trials	Time	Errors	Retraces	Ex. Dist.	Trials	Time	Errors
Control group												
7	258	29	5	56	5	41	0	0	0	80	711	28
8	284	35	5	80	5	48	0	0	0	90	916	30
8	291	36	5	89	5	50	0	0	0	100	925	48
9	295	37	5	92	5	53	0	0	0	100	943	50
9	329	37	6	98	5	54	0	0	0	110	945	50
10	339	40	7	98	5	57	0	0	0	110	949	57
11	381	40	7	111	5	80	0	0	0	110	983	61
11	404	44	9	111	10	94	1	0	2	120	989	66
11	434	47	9	113	10	102	2	0	4	120	1007	69
11	451	50	9	116	10	103	3	0	6	120	1023	72
12	539	59	9	127	10	108	3	0	6	120	1042	75
12	550	59	10	156	10	120	4	0	8	140	1066	79
12	551	62	10	159	10	127	4	0	8	140	1068	79
12	600	68	11	169	10	128	5	0	10	150	1082	80
14	617	69	11	183	10	129	5	0	10	150	1112	82
16	656	69	14	203	10	186	5	0	12	150	1117	84
17	892	81	15	205	10	192	6	0	14	160	1144	91
17	919	92	16	229	10	203	6	1	15	160	1147	91
17	957	110	17	278	10	210	8	1	20	170	1178	93
19	965	113	18	285	15	214	8	2	23	170	1183	96
19	1065	114	19	289	15	219	8	2	27	170	1186	96
20	1089	126	21	314	15	220	10	2	34	170	1204	97
22	1147	127	21	323	15	223	11	2	34	170	1212	99
22	1176	131	21	325	15	226	12	2	35	180	1212	102
22	1278	132	22	334	15	245	15	2	39	180	1214	103
23	1369	134	22	341	15	256	15	2	39	180	1298	109
24	1453	139	23	342	20	263	17	2	40	180	1298	109
24	1454	146	26	374	20	267	22	3	48	190	1337	111
27	1495	153	27	393	20	281	24	3	54	190	1397	116
29	1508	154	28	405	20	282	27	3	56	190	1436	121
30	1523	169	33	427	20	290	27	3	79	190	1460	122
32	1544	172	33	435	20	292	31	3	85	200	1465	126
34	1579	182	36	440	20	331	35	3	88	200	1574	126
37	1590	205	39	482	20	341	37	3	89	200	1642	127
38	1618	207	39	510	20	360	38	4	94	200	1664	132
40	1646	211	42	513	20	370	41	4	102	200	1708	154
41	1864	212	46	553	20	387	46	4	105	220	1788	156
41	2002	248	49	614	25	402	46	5	106	250	1797	156

TABLE 2—*Concluded*

LEARNING MAZE					RELEARNING MAZE					MULTIPLE CHOICE		
Trials	Time	Errors	Retraces	Ex. Dist.	Trials	Time	Errors	Retraces	Ex. Dist.	Trials	Time	Errors
Control group— <i>Concluded</i>												
42	2199	249	50	637	25	413	48	5	111	260	1803	168
51	2265	264	51	660	25	415	48	6	113	270	1920	171
56	2545	276	52	675	25	456	51	8	115	270	1957	171
60	2682	279	57	738	25	474	66	8	140	280	2019	194
70	2831	289	58	752	25	565	77	9	205	290	2083	199
76	3125	314	60	780	30	770	104	13	258	300	2126	223
76	3204	324	67	905	30	907	127	15	311	320	2217	238
79	3343	358	75	914	65	1279	140	16	357	330	2543	265
Median .22.5	1161.5	129	21	324	15	224.5	11.5	2	34.5	175	1212	100.5

Records are included in this table for two rats of the experimental group which failed to learn the maze in 150 trials and which were then discontinued. These two rats are not included in the records for relearning the maze but are included in the multiple choice data. They showed no signs of improvement in the last 50 trials in the maze, and so their complete learning record on the maze, if it had been taken, would have increased all the means of the experimental group. The records of the experimental group show more group variability than the control group as indicated by the relative size of the probable errors and the greater range of scores. With the exception of average time per trial the experimental group have consistently poorer average scores than the control group.

Table 4 shows the differences between the means of the two groups, the probable errors of the differences, the difference over the probable error of the difference, and the chances in 100 that the true difference is greater than zero, for the same measures given in table 3.

The learning curves of the two groups for average errors per trial are similar in shape, the main difference being that the curve for the experimental group extends beyond that of the control

group as more trials were required by some of the experimental group than any of the control group. The control group curve is below that of the experimental group with the exception of seven trials when the experimental group average was better than that of the control group. The curves for average time per

TABLE 3

Maze scores

MEASURE	CONTROL GROUP			EXPERIMENTAL GROUP		
	Mean	P.E.	Range	Mean	P.E.	Range
Trials to learn.....	27.8	.6	7-79	39.8	4.0	7-150
Total time.....	1289.3	85.1	258-3343	1705.2	146.2	105-5818
Average time per trial.....	48.4	2.3	12.1-106.8	47.2	2.6	15-126.3
Total errors.....	141.1	8.6	29-358	200.7	16.9	6-588
Average errors per trial.....	5.5	.23	2.4-11.1	5.6	.29	.9-15.6
Average errors per trial first 7 trials.....	13.9	.83	4.1-40.1	14.7	1.04	.9-36.0
Total retraces.....	26.4	1.9	5-75	33.2	2.8	0-109
Excess distance.....	357.2	23.5	56-914	495.0	36.5	12-1411

TABLE 4

Difference between the means and the reliability of the differences (maze scores)

	DIFFERENCE	P.E. DIFFERENCE	D/P.E. DIFFERENCE	CHANCES
Trials to learn.....	12.0	4.08	2.94	98
Total time.....	415.9	165.7	2.51	95
Average time per trial.....	-1.2	3.43	-.35	59
Total errors.....	59.6	18.94	3.15	98
Average errors per trial.....	.12	0.37	.32	58
Average errors per trial first 7 trials.....	.8	1.33	.60	66
Total retraces.....	6.8	3.4	2.00	91
Excess distance.....	137.8	43.46	3.17	98

trial were also very similar, the control group curve being below that of the experimental group except on fourteen trials.

It has been shown that there is a consistent difference in favor of the control group on total trials to learn the maze, total time, total errors, total retraces and excess distance, all of which measures are probably closely related as time, errors, retraces

and distance are all functions of the number of trials to learn. It has also been shown that there is a small but relatively consistent difference between the groups on average time per trial per individual, and average errors per trial per individual.

3. Results for relearning the maze

Table 5 gives the means, the probable errors of the means, and the range of scores for the two groups on the various measures of the relearning trials.

TABLE 5
Scores for relearning trials

	CONTROL GROUP			EXPERIMENTAL GROUP		
	Mean	P.E.M.	Range	Mean	P.E.M.	Range
Trials to relearn.....	16.4	1.01	5-65	37.3	5.07	5-220
Total time.....	279.0	22.9	41-1279	762.9	138.6	47-5615
Average time per trial.....	15.8	.63	8.2-45.4	17.4	.63	9.4-35.2
Total errors.....	25.7	3.12	0-140	62.8	10.94	0-443
Average errors per trial.....	1.27	.14	0-7.0	1.39	.12	0-4.9
Total retraces.....	3.2	.43	0-16	11.9	2.80	0-127
Excess distance.....	63.1	7.94	0-357	160.9	27.36	0-1159

TABLE 6
Differences between the means and the reliability of the differences (relearning scores)

	DIFFER- ENCE	P.E. DIFFER- ENCE	D/P.E. DIFFER- ENCE	CHANCES
Total trials.....	20.9	5.16	4.05	100
Total time.....	483.9	140.3	3.45	99
Average time.....	1.6	.89	1.80	89
Total errors.....	37.1	11.38	3.26	98.6
Average errors.....	.12	.18	.66	67
Total retraces.....	8.7	2.83	3.07	98
Excess distance.....	97.8	28.48	3.43	99

It may be seen from this table that the control group has a consistently better average record than the experimental group. Also, as before, the experimental group records show more group variability than the control group. The differences between the means and the reliability of the differences are indicated in table 6.

All differences are in favor of the control group, and with the exception of average time per trial per individual and average errors per trial per individual, all differences are more than three times their probable errors.

The results for the relearning trials are similar to the results for the learning of the maze, there being a consistent difference in favor of the control group in terms of trials to relearn the maze,

TABLE 7
Multiple choice scores

	CONTROL GROUP			EXPERIMENTAL GROUP		
	Mean	P.E.M.	Range	Mean	P.E.M.	Range
Total trials.....	184.1	6.4	80-330	281.6	11.7	110-500
Total time.....	1371.5	41.7	711-2543	2281.3	124.6	791-6785
Average time.....	7.4	.18	5.3-13.5	8.1	.31	4.8-17.0
Total errors.....	112.4	2.7	28-265	170.3	7.1	55-290
Average errors.....	.59	.013	.28-.83	.64	.014	.44-.93

TABLE 8
Differences between the means and the reliability of the differences (multiple choice scores)

	DIFFER- ENCE	P.E. DIFFER- ENCE	D/P.E. DIFFER- ENCE	CHANCES
Total trials.....	97.5	13.37	7.29	100
Total time.....	909.8	131.4	6.92	100
Average time.....	.7	.36	1.94	91
Total errors.....	57.9	7.59	7.63	100
Average errors.....	.05	.019	2.63	96

and a small difference in average time per trial per individual and average number of errors per trial per individual.

4. Results for the multiple choice problem

Table 7 indicates the mean scores, the probable errors of the means, and the range of scores for the two groups on the various measures of the multiple choice problem.

The differences between the groups and the reliability of the differences are given in table 8.

There is then a consistent difference in favor of the control group on all the measures of the multiple choice problem, and this difference is reliable in the case of trials to learn, total time and total errors, and very nearly reliable in the case of average time per trial per individual and average errors per trial per individual.

The curves for average errors per trial for the two groups are very similar in configuration, the control group curve being below that of the experimental group for all trials except six. The learning curves for average time per trial for the two groups are again similar in shape except that the curve for the control group drops off more rapidly than the curve for the experimental group. The experimental group curve equals or is better than the control group curve on only two trials.

On all measures used the control group had better average scores than the experimental group, and in most cases the differences were statistically reliable.

5. Consistency of the individual scores

In a later section individual learning scores are correlated with weight at different ages, so some measure of the consistency of the individual records was desired. This was achieved by correlating the scores on the odd trials with the scores on the even trials of the first twenty maze trials of 44 cases selected from both groups. The coefficients obtained in this way were as follows—time $+.68 \pm .05$, errors $+.65 \pm .06$, excess distance $+.63 \pm .06$. These coefficients seem to indicate a fair degree of consistency of individual performance on the maze; however when the sum of the errors on the first ten trials was correlated with the total errors on the second ten trials of the same subjects as before, the resulting coefficient was $+.03 \pm .10$. And also when the total errors on the first five trials was correlated with the number of trials to learn for all the animals of both groups the coefficient was $+.10 \pm .07$. On the multiple choice problem the records for 100 trials were correlated with the records for another 100 trials in alternate blocks of 10 trials for 37 cases selected from both groups. The coefficients were $+.91 \pm .02$ for total time and

$+.94 \pm .01$ for total errors. These coefficients indicate a high degree of consistency of individual performance in the multiple choice problem. However, if the consistency were calculated by other methods the resulting coefficients may be small as it was shown above that the size of the consistency coefficient for the maze depended upon the method of calculation.

Maze records were correlated with multiple choice records and relearning records. The product moment coefficients obtained are given in table 9. This table of coefficients indicates that there is probably no significant relation between the scores of the different problems used.

TABLE 9
Intercorrelations of learning scores

	N	r	P.E.r
Maze trials versus multiple choice trials.....	83	-.04	.07
Maze time versus multiple choice time.....	83	-.15	.07
Maze errors versus multiple choice errors.....	83	-.06	.07
Maze trials versus relearning trials.....	81	-.06	.08
Maze time versus relearning time.....	81	-.13	.07
Maze errors versus relearning errors.....	81	-.10	.07

6. Weight and learning scores

As one of the more obvious signs of vitamin B deficiency is a loss in the rate of increase of weight, an attempt was made to relate weight and learning scores. The following weight records were correlated by the rank position method with the different maze and multiple choice measures—weight at four weeks, the average weight for weeks 4 to 8 inclusive, the average weight for weeks 27 to 31 inclusive, the average weight for weeks 4 to 31 inclusive, and the weight at the thirty-first week. Because of the great sex difference in weight the sexes were correlated separately, and the two groups were also treated separately as it was desired to compare the coefficients of the two groups. Table 10 summarizes the results of these correlations.

With the experimental group the ten coefficients of correlation between weight and maze trials are all positive while only two

of the ten coefficients of the control group are positive, so it may be said that there is a slight positive correlation of weight and trials to learn the water maze with the experimental group but no such relationship in the control group. The same type of relationship is noticed with trials to learn the multiple choice problem and weight but to a lesser degree than with the maze. The coefficients for average time and average errors on the maze are predominately negative for the experimental group but about equally positive and negative for the control group. It may be

TABLE 10
Correlation of weight and learning scores

Weight at.....	4 weeks		4-8 wks.		27-31 wks.		4-31 wks.		31 wks.	
Sex.....	F	M	F	M	F	M	F	M	F	M
Experimental group										
Maze trials.....	+ .19	+ .62	+ .20	+ .58	+ .15	+ .26	+ .22	+ .46	+ .24	+ .31
Maze average time....	- .36	- .11	- .39	+ .09	- .01	- .37	- .19	- .23	- .01	- .34
Maze average errors...	- .30	- .04	- .27	+ .06	+ .10	- .28	- .01	- .21	+ .07	- .28
M.C. trials.....	+ .04	- .01	- .04	- .09	+ .20	+ .34	+ .34	+ .16	+ .22	+ .28
M.C. average time....	+ .26	- .31	+ .14	- .17	- .59	- .38	- .59	- .41	- .74	- .44
M.C. average errors...	+ .65	+ .22	+ .61	- .07	- .10	+ .07	+ .20	+ .03	- .15	+ .09
Control group										
Maze trials.....	- .13	+ .25	- .08	+ .27	- .08	- .31	- .08	- .12	- .09	- .25
Maze average time....	- .01	+ .44	- .17	+ .27	+ .04	+ .02	- .08	- .02	- .02	- .01
Maze average errors...	- .12	+ .31	- .18	+ .12	+ .18	+ .05	+ .06	+ .07	+ .13	- .03
M.C. trials.....	- .19	- .14	+ .06	- .37	+ .35	- .13	+ .31	- .21	+ .37	- .17
M.C. average time....	+ .50	- .08	+ .25	+ .06	- .15	+ .19	- .17	+ .03	- .06	+ .10
M.C. average errors...	- .15	+ .03	+ .02	- .26	- .01	+ .20	+ .04	- .01	+ .01	+ .15

concluded therefore that there is a slight positive correlation between weight and trials to learn the maze and multiple choice problem, the heavier animals tending to be the better learners, with vitamin B deficient rats, but that such a correlation is probably absent with the normal controls. This corresponds with the results given above, namely, that the animals of the control group which were heavier on the average than the experimental group were also the better learners.

IV. SUMMARY

1. Seventy-six rats were nursed from birth for four weeks by mothers whose diet was deficient in vitamin B. Of this number thirty-nine died. The remaining thirty-seven rats were given a normal diet from four weeks of age on. Seventy-three rats were used as normal controls, twenty-five of which died and two were discarded.

2. The deficient group was considerably less in weight at every stage of the experiment than the control group.

3. The deficient group had a slightly higher activity score than the control group.

4. There was practically no difference between the groups in preliminary swimming as measured by the time taken to swim out of a tank on twenty trials.

5. Beginning when the animals were eight weeks of age, both groups were given learning trials in a water maze, relearning trials in the same maze two months after completing learning, and trials in a multiple choice problem using the escape-from-water incentive.

6. Intercorrelations between the measures of the three problems were small and in every case minus.

7. In trials to learn the maze and trials to relearn the same maze after two months the control group had consistently better average scores than the experimental group.

8. There were only slight differences between the groups in average time per trial (speed of swimming) and average errors per trial in learning and relearning the maze.

9. There was a consistent difference in favor of the control group on all scores of the multiple choice problem, but this difference was greater in the case of trials to learn than it was for average time per trial and average errors per trial.

10. The experimental group showed more group variability than the control group on all learning measures as shown by the relative size of the probable errors of the means, and the range of scores.

11. There was some slight indication that the heavier animals

of the experimental group tended to be better learners than the lighter animals of the same group. There was no such relationship noticeable in the control group. The control group rats which were heavier on the average than the members of the experimental group were also the better learners.

V. CONCLUSIONS AND DISCUSSION

The process of removing vitamin B from the diet of the nursing mothers of the experimental group caused severe physiological effects on the developing animals. This effect is apparent in the weight curves. The slower rate of growth of the deficient animals probably had some adverse effect on the developing nervous system. Histological examination of the nervous systems of the eight best learners of the control group and the eight worst learners of the experimental group is being made and the results will be reported later. It is assumed that learning is closely bound up with the functioning of the central nervous system and that if the nervous system is affected by the vitamin B deficiency this effect might be noticeable in the learning scores of the animals.

Maurer and Tsai demonstrated that early deficiency of vitamin B did result in poorer learning scores on a food incentive maze. The present investigation has shown that this effect is also present with a different maze pattern, a different incentive, namely, escape-from-water, and with a different type of learning test—multiple choice—as well as in relearning the maze after two months. It may be concluded therefore, that vitamin B deficiency during the first few weeks of the life of the rat has an adverse effect on the learning ability of some animals even if vitamin B is given in abundance during the time of the learning tests.

The effect of vitamin B deficiency on learning would probably be greater if several factors were not in operation. It is possible that the vitamin B depletion was not complete under the conditions of the experiment. The mothers of the experimental group were fed on a normal diet previous to the birth of the experimental litters, and it is possible therefore that the rats when born would have a certain amount of vitamin B in storage. Also as has been

noted above, animals differ greatly in their ability to withstand the effects of dietary deficiencies. Chance factors in the learning situation, individual differences in learning ability, differences in distractibility and other factors may have contributed to the result as well. These facts might explain why only some of the animals of the experimental group seemed to be affected while others seemed to be as good learners as any of the normal control group. Another factor, also mentioned previously, which should be considered in this connection, is that various organs of the body are affected in differing degrees by dietary deficiencies, the brain being affected less than some other parts of the body. It is possible then, that although the effects of vitamin B deficiency were noticeable in the weights of the animals, this difference in weights may not be an indication of very great effects on the nervous system. That vitamin B deficiency does have some detrimental effect on the central nervous system, however, was demonstrated by Stern and Findlay (15). Lashley (7) has shown that comparatively large amounts of nervous tissue may be destroyed in the rat without very great behavior symptoms. It is possible then, that the effects of vitamin B deficiency on the nervous system of the rat may not be severe enough to cause very great differences in learning ability. However, that there is some effect on the rate of learning is evident from the results of this experiment as well as that of Maurer and Tsai. Also, the results of this experiment as well as those of Maurer and Tsai's experiment when compared with the results of the experiments of Fritz and Frank demonstrate that the effect of early deficiency in vitamins is probably greater than a like deficiency with mature rats.

It is, of course, difficult to extend the results of this experiment to the realm of human learning. There are several suggestions in the literature that the effect of dietary deficiency may be less with human subjects than with lower animals. Nevertheless it would seem that if vitamin B deficiency after birth with rats is effective in reducing learning ability, it is at least possible that such deficiency in the developing human being might also be sufficient to cause a decreased ability to adjust to complex

situations. Due to the multiplicity of complicating factors it is much more difficult to detect with human subjects the effects of differences in diets, so for the time being it seems worth while to continue using animal subjects in such experiments, and to accept as at least a possibility that the effects demonstrated in lower animals may also be true of human subjects. Certainly the abundance of evidence collected from the physiological standpoint seems to point to the conclusion that dietary differences have wide spread effects on the human organism, and no doubt some of these effects are mirrored in behavior.

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